(1390 REV. 5-93) US DEPT. OF COMMERCE PATENT & TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER
110530

U.S. APPLICATION NO.
(if known, sec 37 C.F.R.1.5)
09/936921

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR00/00754	March 24, 2000	March 26, 1999

TITLE OF INVENTION
DIAGNOSIS OF WHIPPLE'S DISEASE

APPLICANT(S) FOR DO/EO/US
Didier RAOULT, Bernard LA SCOLA, Marie-Laure BIRG, Florence FENOLLAR

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)

6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. ☒ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.

    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ Entitlement to small entity status is hereby asserted.

16. ☐ Other items or information:

U.S. APPLICATION NO. (if known, see 37 C.F.R. 1.5) 09/936921	INTERNATIONAL APPLICATION NO. PCT/FR00/00754	ATTORNEY'S DOCKET NUMBER 110530

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National fee (37 CFR 1.492(a)(1)-(5)):		
Search Report has been prepared by the EPO or JPO $860.00		
International preliminary examination fee paid to USPTO (37 CFR1.482) $690.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) $710.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....$1,000.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).....$ 100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$860.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	28 - 20 =	8	X $ 18.00	$144.00	
Independent Claims	3 - 3 =	0	X $ 80.00	$	
Multiple dependent claim(s)(if applicable)			+ $270.00	$	
TOTAL OF ABOVE CALCULATIONS =				$1004.00	
Reduction by 1/2 for filing by small entity, if applicable.			-	$	
SUBTOTAL =				$1004.00	
Processing fee of $130.00 for furnishing the English translation later than ☐ 20 ☐ 30 month from the earliest claimed priority date (37 CFR 1.492(f)).			+	$	
TOTAL NATIONAL FEE =				$1004.00	
				Amount to be refunded	$
				Charged	$

a. ☒ Check No. 122918 in the amount of $1004.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Director is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Deposit Account No. 15-0461. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:
OLIFF & BERRIDGE, PLC
P.O. Box 19928
Alexandria, Virginia 22320

Date: September 20, 2001

NAME: William P. Berridge
REGISTRATION NUMBER: 30,024

NAME: Melanie L. Mealy
REGISTRATION NUMBER: 40,085

(1390 Rev.10-00)

SEQUENCE LISTING

Key

Diagnostic de la maladie de Whipple = Diagnosis of Whipple's disease
ADN = DNA
Séquence artificielle = Artificial sequence
Description de la séquence artificielle: oligonucléotide = Description of the artificial sequence: oligonucleotide

Key to Figures

FEUILLE DE REMPLACEMENT (REGLE 26) = REPLACEMENT SHEET (RULE 26)

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Didier RAOULT, Bernard LA SCOLA, Marie-Laure BIRG and Florence FENOLLAR

BOX: SEQUENCE

US National Stage of PCT/FR00/00754

Filed: September 20, 2001

Docket No.: 110530

For: DIAGNOSIS OF WHIPPLE'S DISEASE

PRELIMINARY AMENDMENT

Director of the U.S. Patent and Trademark Office
Washington, D. C. 20231

Sir:

Prior to initial examination, and after entry of the annexes to the IPER, please amend the above-identified application as follows:

IN THE SPECIFICATION:

At the end of the application, please replace the current Sequence Listing with the attached paper and computer-readable Sequence Listing.

IN THE CLAIMS:

Please replace claims 3-16 and 18-24 as follows:

3. (Amended) Bacterium according to claim 1, wherein said bacterium is deposited in the CNCM of the Institut Pasteur under the number I-2202.

4. (Amended) Antigen of a bacterium according to claim 1.

5. (Amended) Antigen according to claim 4, wherein said antigen is a protein selected from those with molecular weights of about 35, 50, 60, 100 and 200 kD determined in Figures 2 and 3 by polyacrylamide gel electrophoresis using the Western blotting technique.

6. (Amended) Specific antibody directed against a bacterium *Tropheryma whippelii* responsible for Whipple's disease or an antigen of said bacterium.

7. (Amended) Antibody according to claim 6, wherein it is a polyclonal antibody of animal origin, preferably a mouse immunoglobulin.

8. (Amended) Antibody according to claim 6, wherein it is a monoclonal antibody.

9. (Amended) Antibody according to claim 8, wherein it is a monoclonal antibody produced by a hybridoma deposited in the CNCM of the Institut Pasteur under the registration number I-2411.

10. (Amended) Antigen according to claim 5, wherein said antigen is a protein of 200 kD which reacts with a specific monoclonal antibody directed against a bacterium *Tropheryma whippelii* responsible for Whipple's disease or an antigen of said bacterium, said antibody being produced by a hybridoma deposited in the CNCM of the Institut Pasteur under the registration number I-2411.

11. (Amended) Method for the *in vitro* diagnosis of diseases associated with infections caused by the bacterium *Tropheryma whippelii*, comprising bringing serum or any other biological fluid of a patient into contact with the bacterium of claim 1.

12. (Amended) Method for *in vitro* diagnosis of the disease associated with infections caused by *Tropheryma whippelii* bacteria, comprising bringing serum or any other biological fluid of a patient into contact with the antibody of claim 6.

13. (Amended) Method for the *in vitro* serological diagnosis of Whipple's disease, comprising the steps which consist essentially of detecting an immunological reaction between an antibody according to claim 6 and an antigen of a bacterium *Tropheryma whippelii* responsible for Whipple's disease.

14. (Amended) Method for the *in vitro* serological diagnosis of Whipple's disease, comprising the step which consists essentially of detecting an immunological reaction between a human immunoglobulin which recognizes bacterium *Tropheryma whippelii* responsible for Whipple's disease and an antibody specific for said human immunoglobulin.

15. (Amended) Method of serological diagnosis according to claim 14 comprising the following steps:

- depositing a solution containing a bacterium *Tropheryma whippelii* responsible for Whipple's disease, in or on a solid support;
- introducing the test serum or biological fluid into or onto said support;
- introducing a solution of a labeled antibody specific for a human immunoglobulin which recognizes said bacterium, into or onto the support;
- observing an incubation period;

- rinsing the solid support; and
- detecting said immunological reaction.

16. (Amended) Kit for the *in vitro* detection of Whipple's disease by the method of claim 13, essentially comprising the following components:

- a solution containing a bacterium *Tropheryma whippelii* responsible for Whipple's disease or an antigen of said bacterium; and/or
- a solution containing at least one specific antibody directed against a bacterium *Tropheryma whippelii* responsible for Whipple's disease or against an antigen of said bacterium; and/or
- a solution containing at least one antibody specific for a human immunoglobulin, which recognizes a bacterium *Tropheryma whippelii* responsible for Whipple's disease.

18. (Amended) Fragment of the *rpoB* gene of the bacterium *Tropheryma whippelii* according to claim 1, wherein said fragment comprises the nucleotide sequence SEQ ID NO: 3.

19. (Amended) Oligonucleotide comprising a sequence specific for the *rpoB* gene of the bacterium *Tropheryma whippelii* according to claim 1, said specific sequence comprising at least 12 consecutive nucleotide units included in the sequence SEQ ID NO: 3.

20. (Amended) Single-stranded oligonucleotide according to claim 19 selected from oligonucleotides having a sequence of at least 12 consecutive nucleotide units included in one of the sequences of SEQ ID NOs: 4 and 5, and from the oligonucleotides complementary to these oligonucleotides.

21. (Amended) Oligonucleotide according to claim 19, wherein it consists of the sequences SEQ ID NOs: 4 and 5.

22. (Amended) Probe for detecting *Tropheryma whippelii* bacteria in a biological sample, wherein said probe comprises a sequence according to claim 18.

23. (Amended) Process for determining the presence or absence of a *Tropheryma whippelii* bacterium in a sample which contains or may contain nucleic acids of at least one such bacterium, wherein said sample is brought into contact with at least one probe according to claim 22 and the formation or absence of formation of a hybridization complex between said probe and the nucleic acid of the sample is then determined.

24. (Amended) Nucleotide primer which can be used for synthesizing the *rpoB* gene of *Tropheryma whippelii* in the presence of a polymerase, wherein said primer comprises an oligonucleotide according to claim 19.

Please add new claims 25-28 as follows:

--25. Method for *in vitro* diagnosis of the disease associated with infections caused by *Tropheryma whippelii* bacteria, comprising bringing serum or any other biological fluid of a patient into contact with the antigen of claim 4. --

--26. Kit for the *in vitro* detection of Whipple's disease by the method of claim 14, essentially comprising the following components:

- a solution containing a bacterium *Tropheryma whippelii* responsible for Whipple's disease or an antigen of said bacteria; and/or
- a solution containing at least one specific antibody directed against a bacterium *Tropheryma whippelii* responsible for Whipple's disease or against an antigen of said bacteria; and/or
- a solution containing at least one antibody specific for a human immunoglobulin, said human immunoglobulin recognizes a bacterium *Tropheryma whippelii* responsible for Whipple's disease. --

--27. Probe for detecting *Tropheryma whippelii* bacteria in a biological sample, wherein said probe comprises an oligonucleotide according to claim 19. --

--28.. Process for determining the presence or absence of a *Tropheryma whippelii* bacterium in a sample which contains or may contain nucleic acids of at least one such bacterium, wherein said sample is brought into contact with at least one probe according to claim 27 and the formation or absence of formation of a hybridization complex between said probe and the nucleic acid of the sample is then determined. --

REMARKS

Claims 1-28 are pending. By this Preliminary Amendment, the sequence listing is replaced; claims 3-16 and 18-24 are amended; and claims 25-28 are added.

The attached Appendix includes marked-up copies of each rewritten claim (37 C.F.R. 1.121(c)(1)(ii)).

The attached paper copy and computer-readable copy of the Sequence Listing are submitted in compliance with 37 C.F.R. §§1.821-1.825. The contents of the paper copy and the computer-readable copy of the Sequence Listing are the same. No new matter is added. Support for the information provided in the Sequence Listing can be found in the original Sequence Listing.

Early and favorable consideration on the merits is respectfully requested.

Respectfully submitted,

William P. Berridge
Registration No. 30,024

Melanie L. Mealy
Registration No. 40,085

WPB:MLM/zmc

Attachments:
Appendix
Sequence Listing (paper and computer-readable copies)

Date: September 20, 2001

OLIFF & BERRIDGE, PLC
P.O. Box 19928
Alexandria, Virginia 22320
Telephone: (703) 836-6400

DEPOSIT ACCOUNT USE AUTHORIZATION
Please grant any extension necessary for entry;
Charge any fee due to our Deposit Account No. 15-0461

APPENDIX

Changes to Specification:

The Sequence Listing is replaced.

Changes to Claims:

Claims 25-28 are added.

The following are marked-up versions of the amended claims:

3. (Amended) Bacterium according to claim 1 ~~or 2~~, ~~characterized in that it~~ wherein said bacterium is deposited in the CNCM of the Institut Pasteur under the number I-2202.

4. (Amended) Antigen of a bacterium according to claim 1 ~~one of claims 1 to 3~~.

5. (Amended) Antigen according to claim 4, ~~characterized in that it~~ wherein said antigen is a protein selected from those with molecular weights of about 35, 50, 60, 100 and 200 kD determined in Figures 2 and 3 by polyacrylamide gel electrophoresis using the Western blotting technique.

6. (Amended) Specific antibody directed against ~~the~~ a bacterium *Tropheryma whippelii* responsible for Whipple's disease or an antigen of ~~the~~ said bacterium ~~according to one of claims 1 to 5~~.

7. (Amended) Antibody according to claim 6, ~~characterized in that~~ wherein it is a polyclonal antibody of animal origin, preferably a mouse immunoglobulin.

8. (Amended) Antibody according to claim 6, ~~characterized in that~~ wherein it is a monoclonal antibody.

9. (Amended) Antibody according to claim 8, ~~characterized in that~~ wherein it is a monoclonal antibody produced by a hybridoma deposited in the CNCM of the Institut Pasteur under the registration number I-2411.

10. (Amended) Antigen according to claim 5, ~~characterized in that it~~ wherein said antigen is a protein of 200 kD which reacts with ~~an~~ specific monoclonal antibody ~~according to claim 9~~directed against a bacterium *Tropheryma whippelii* responsible for Whipple's disease or an antigen of said bacterium, said antibody being produced by a hybridoma deposited in the CNCM of the Institut Pasteur under the registration number I-2411.

11. (Amended) ~~Use of a bacterium according to any one of claims 1 to 3 or an antigen according to claim 4, 5 or 10~~ Method for the *in vitro* diagnosis of diseases associated

with infections caused by the bacterium *Tropheryma whippelii*, comprising bringing serum or any other biological fluid of a patient into contact with the bacterium of claim 1.

12. (Amended) ~~Use of an antibody according to one of claims 6 to 9~~ Method for *in vitro* diagnosis of the disease associated with infections caused by *Tropheryma whippelii* bacteria, comprising bringing serum or any other biological fluid of a patient into contact with the antibody of claim 6.

13. (Amended) Method for the *in vitro* serological diagnosis of Whipple's disease, comprising the steps which consist essentially ~~in~~ of detecting an immunological reaction between an antibody ~~specific for the bacterium~~ according to ~~one of~~ claims 6 ~~to 9~~ and an antigen of ~~said~~ a bacterium ~~according to one of claims 4, 5 and 10~~*Tropheryma whippelii* responsible for Whipple's disease.

14. (Amended) Method for the *in vitro* serological diagnosis of Whipple's disease, comprising the step which consists essentially ~~in~~ of detecting an immunological reaction between ~~an antibody specific for~~ a human immunoglobulin which recognizes ~~said~~ bacterium ~~according to one of claims 1 to 3~~ *Tropheryma whippelii* responsible for Whipple's disease and ~~a~~ an antibody specific for said human immunoglobulin ~~which recognizes said bacterium according to claims 1 to 5~~.

15. (Amended) Method of serological diagnosis according to claim 14 comprising the following steps:

- depositing a solution containing ~~the~~ a bacterium ~~as defined in claims 1 to 3~~*Tropheryma whippelii* responsible for Whipple's disease, in or on a solid support;
- introducing the test serum or biological fluid into or onto said support;
- introducing a solution of a labeled antibody specific for a human immunoglobulin which recognizes said bacterium, into or onto the support;
- observing an incubation period;
- rinsing the solid support; and
- detecting said immunological reaction.

16. (Amended) Kit for the *in vitro* detection of Whipple's disease by the method of ~~one of~~ claims 13 ~~to 15~~, essentially comprising the following components:

- a solution containing ~~the~~ a bacterium *Tropheryma whippelii* responsible for Whipple's disease or an antigen ~~as defined in claims 1 to 5 and 10~~of said bacterium; and/or

- a solution containing at least one specific antibody ~~according to one of claims 6 to 9~~directed against a bacterium *Tropheryma whippelii* responsible for Whipple's disease or against an antigen of said bacterium; and/or

- a solution containing at least one antibody specific for a human immunoglobulin, which recognizes ~~said~~ a bacterium ~~according to claims 1 to 3~~*Tropheryma whippelii* responsible for Whipple's disease.

18. (Amended) Fragment of the *rpoB* gene of the bacterium *Tropheryma whippelii* according to ~~one of~~ claims 1 ~~to 3~~, ~~characterized in that it~~ wherein said fragment comprises the nucleotide sequence SEQ ID ~~N°~~NO: 3.

19. (Amended) Oligonucleotide comprising a sequence specific for the *rpoB* gene of the bacterium *Tropheryma whippelii* according to ~~one of~~ claims 1 ~~to 3~~, said specific sequence comprising at least 12 consecutive nucleotide units included in the sequence SEQ ID ~~N°~~NO: 3.

20. (Amended) Single-stranded oligonucleotide according to claim 19 selected from oligonucleotides having a sequence of at least 12 consecutive nucleotide units included in one of the sequences ~~to~~ of SEQ ID ~~N°~~NOs: 4 and 5, and from the oligonucleotides complementary to these oligonucleotides.

21. (Amended) Oligonucleotide according to claim 19 ~~or 20~~, ~~characterized in that~~ wherein it consists of the sequences SEQ ID ~~N°~~NOs: 4 and 5.

22. (Amended) Probe for detecting *Tropheryma whippelii* bacteria in a biological sample, ~~characterized in that it~~ wherein said probe comprises a sequence according to claim 18 ~~or an oligonucleotide according to one of claims 19 to 21~~.

23. (Amended) Process for determining the presence or absence of a *Tropheryma whippelii* bacterium in a sample which contains or may contain nucleic acids of at least one such bacterium, ~~characterized in that~~ wherein said sample is brought into contact with at least one probe ~~...~~ according to claim 22 and the formation or absence of formation of a hybridization complex between said probe and the nucleic acid of the sample is then determined.

24. (Amended) Nucleotide primer which can be used for synthesizing the *rpoB* gene of *Tropheryma whippelii* in the presence of a polymerase, ~~characterized in that it~~ wherein said primer comprises an oligonucleotide according to claims 19 ~~to 21, preferably an oligonucleotide comprising one of the sequences SEQ ID N° 4 and SEQ ID N° 5~~.

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Didier RAOULT et al.

Application No.: 09/936,921

Filed: September 24, 2001

For: DIAGNOSIS OF WHIPPLE'S DISEASE

BOX: SEQUENCE

Docket No.: 110530

SUPPLEMENTAL PRELIMINARY AMENDMENT

Director of the U.S. Patent and Trademark Office
Washington, D.C. 20231

Sir:

In reply to the Notification of Missing Requirements mailed March 15, 2002, please amend the above-identified application as follows:

IN THE SPECIFICATION:

At the end of the application, please replace the current Sequence Listing with the attached paper and computer-readable Sequence Listing.

REMARKS

Claims 1-28 are pending.

The attached paper copy and computer-readable copy of the Sequence Listing are submitted in compliance with 37 C.F.R. §§1.821-1.825. The contents of the paper copy and the computer-readable copy of the Sequence Listing are the same. No new matter is added. Support for the information provided in the Sequence Listing can be found in the original Sequence Listing.

Early and favorable consideration on the merits is respectfully requested.

Respectfully submitted,

William P. Berridge
Registration No. 30,024

Melanie L. Mealy
Registration No. 40,085

WPB:PAC/jca

Attachment:
Sequence Listing (paper and computer-readable copies)

Date: May 15, 2002

OLIFF & BERRIDGE, PLC
P.O. Box 19928
Alexandria, Virginia 22320
Telephone: (703) 836-6400

DEPOSIT ACCOUNT USE AUTHORIZATION
Please grant any extension necessary for entry;
Charge any fee due to our Deposit Account No. 15-0461

Diagnosis of Whipple's disease

The present invention relates to the field of diagnostics. More precisely, the invention relates to a method for the *in vitro* serological diagnosis of Whipple's disease and to a device for carrying out this method. The invention further relates to a kit for *in vitro* detection of the bacterium responsible for Whipple's disease.

The present invention further relates to the field of the techniques of detection and/or amplification and sequencing with the aid of oligonucleotide probes or primers and to their application to tests for detecting the presence of bacteria of the species *Tropheryma whippelii* or to their identification.

Whipple's disease is a disease which manifests itself in a variety of forms. The most classic form is that of a fever with chronic diarrhea leading to weight loss, but this disease is also capable of giving rise to chronic articular symptoms, cerebral symptoms with dementia and also cardiac symptoms, particularly endocarditis with negative hemoculture.

Since it was first described in 1907, Whipple's disease has been understood to involve the existence of a bacterium associated with "intestinal lipodystrophy" due to the observation of numerous microorganisms after the silver staining of a mesenteric ganglion (Whipple, Bull. John Hopkins Hosp. 1907; 18: 328-391). Demonstration of the non-specific PAS-positive (PAS = periodic acid-Schiff) character of this bacterium and subsequent observations by electron microscopy confirm the presence of an intracellular bacterial species of Gram-positive structure (Chears et al., Gastroenterology 1961; 41: 129-138). The universal molecular tool 16S rRNA made it possible to confirm this hypothesis, specifying the phylogenic taxonomy of this novel bacterial species and assigning to it the provisional name *Tropheryma whippelii* to evoke the idea of intestinal malabsorption and to honor the discoverer of the complaint (Relman et al., N. Engl. J. Med. 1992; 327: 293-301). The direct sequencing of 721 bases of an amplified fragment from a biopsy of one patient's small intestine (Wilson et al., Lancet 1991; 338: 474-475) and then from another patient's ganglion (Wilson et al., ASM News 1992; 58: 318-321) confirms the novelty of the bacterial species associated with Whipple's disease. The sequencing by Relman et al. (op. cit.) of 1321 bases in one sample, representing 90% of the gene, and of a fragment of 284 bases in four other patients made it possible to confirm that the bacterial species associated with Whipple's

disease represented a novel species, and to specify its taxonomic position in the phylum of the actinomycetes, i.e. the bacteria of Gram-positive structure with a high content of guanosine plus cytosine, representing a new branch relatively close to two species known in human pathology, namely *Actinomyces pyogenes* and *Rothia dentocariosa*.

The disease is currently diagnosed by microscopic observation, after staining, of a smear obtained from a biopsy, or by amplification and sequencing of the universal genetic tool 16S rRNA (Relman et al., op. cit.).

Hitherto, it has in fact been impossible to isolate and cultivate the bacterium responsible for Whipple's disease in a manner suitable for performing serological tests.

Contrary to all expectation, the Applicant has developed a method of culturing the bacterium responsible for Whipple's disease.

The inventors have discovered that the cell culture which enables the bacterium *Tropheryma whippelii* to be isolated and multiplied must have both a long lifetime and a slow multiplication time. They have in fact demonstrated that the doubling time of the bacterium is very long (18 days). Preferably, the primary culture should even be carried out directly on immortalized cells.

Earlier studies carried out on primary cultures of human blood monocytes (SHOEDON et al., "Journal of Infectious Diseases", volume 176, number 3, 1997, pages 672-677) could not be used as a basis for establishing the bacterium *Tropheryma whippelii* in culture in such a way that it multiplied, because the mean lifetime of these monocytes is only 30 days, which is insufficient in view of the doubling time of the bacterium.

In addition, if the cells multiply too rapidly relative to the growth time of the bacterium, they cannot be cultivated because a dilution effect takes place and it becomes impossible to segregate the infected cells from the non-infected cells.

In one advantageous embodiment, the inventors used immortalized fibroblasts. These fibroblasts spread out over the bottom of the culture dish, cease multiplying when they have filled the whole of the cellular mat, but can be kept alive for several months under these conditions.

More precisely, the method of bacterial isolation and culture described in detail in Examples 1 and 2 below comprises inoculating human fibroblasts of the HEL line with a ground heart valve preparation in MEM. The bacterium

responsible for Whipple's disease was isolated and established in culture after incubation for a minimum of two months, the culture medium being replaced regularly. The expression "established in culture" is understood as meaning that the bacterium is obtained reproducibly and multiplies over time, especially via successive subcultures on a cell culture.

The present invention therefore relates to the bacterium isolated and established in this way as an antigen source. This bacterium has been deposited in the CNCM (Paris - France) under no. I-2202 and under the identification reference TWIST-Marseille.

The present invention further relates to an antigen of the bacterium according to the invention. More particularly, the present invention relates to an antigen which is a protein selected from those with molecular weights of about 10, 20, 35, 50, 60, 80, 100, 120, 150, 170 and 200 kD determined by polyacrylamide gel electrophoresis using the SDS-PAGE technique and by Western blotting.

The present invention further relates to a specific antibody directed against the bacterium or an antigen according to the invention, more particularly a polyclonal antibody of animal origin, especially a mouse or rabbit immunoglobulin, or a monoclonal antibody, especially a monoclonal antibody produced by the hybridoma deposited in the CNCM of the Institut Pasteur under the registration number I-2411 and under the identification reference TW 17G2.

The present invention further relates to the detection of an antibody specific for a human immunoglobulin which recognizes said bacterium, preferably IgG, IgM or IgA, and more particularly an animal immunoglobulin, especially an anti-human goat immunoglobulin.

The present invention further relates more particularly to an antigen, characterized in that it is a protein of 200 kD which reacts with a monoclonal antibody produced by the above-mentioned hybridomas according to the invention.

The present invention further relates to the use of a bacterium, an antigen of the bacterium or a specific antibody according to the invention in a method for the *in vitro* diagnosis of diseases associated with infections caused by the bacterium *Tropheryma whippelii*, and to a method for serological diagnosis of the infection caused by the bacterium *Tropheryma whippelii* according to the invention, which comprises bringing the serum or any other biological fluid of a patient into contact with said bacterium and detecting an immunological reaction.

More particularly, the present invention relates to a method for the *in vitro* serological diagnosis of infections caused by *Tropheryma whippelii*, wherein the bacterium according to the invention, an antigen of the bacterium according to the invention or a specific antibody according to the invention is brought into contact with a sample taken from the patient and consisting of a human serum, biological fluid or swab.

The method according to the invention comprises the step which consists essentially in detecting an immunological reaction between an antibody specific for the bacterium according to the invention and an antigen of said bacterium, or between an antibody specific for an immunoglobulin according to the invention which recognizes said bacterium and a said human immunoglobulin which recognizes said bacterium.

The present invention further relates to a method for the *in vitro* serological diagnosis of Whipple's disease, which comprises bringing the serum or any other biological fluid of a patient into contact with the bacterium as defined above, and detecting the immunological reaction.

In one embodiment, the diagnostic method according to the invention comprises:

- depositing a solution of bacterium according to the invention, especially 0.5 to 5 µl and preferably 1 µl of said solution containing said bacterium, in or on a solid support;
- introducing the test serum or biological fluid, preferably diluted, into or onto said support;
- introducing a solution of a labeled antibody, especially an anti-human animal immunoglobulin specific for the human immunoglobulin, especially of the IgG, IgM or IgA type, which recognizes said bacterium, into or onto the support;
- observing an incubation period;
- rinsing the solid support if appropriate; and
- actually detecting the immunological reaction especially between a human antibody which recognizes said bacterium and said anti-human immunoglobulin.

Advantageously, the diagnostic method of the invention involves an enzyme immunoassay of the ELISA type or an immunofluorescent assay. More particularly, the method according to the invention comprises:

- depositing a solution of bacterium, isolated and established as indicated

above, in or on a solid support;

- introducing the diluted test serum or biological fluid into or onto said support;

- introducing a solution of labeled anti-human immunoglobulin into or onto the support;

- observing an incubation period;

- rinsing the solid support if appropriate; and

- actually detecting the immunological reaction.

The solid support used can be any device suitable for handling cellular and bacterial suspensions, especially tubes, glass slides, bijoux tubes or rigid microtiter plates made of polyethylene, polystyrene, polyvinyl chloride or nitrocellulose and containing microwells; glass slides are preferred.

The antibody detected is an immunoglobulin, especially of the G, M or A type, which is specific for the bacterium responsible for Whipple's disease. The type of labeling used for the anti-human immunoglobulin is enzymatic, radioactive or fluorescent labeling, this last type of labeling being preferred.

The expression "fluorescent labeling" means that the antibody has been rendered fluorescent with an appropriate fluorescent agent, such as fluorescein iso(thio)cyanate, combined with an animal immunoglobulin which recognizes the human antibody.

The expression "radioactive labeling" means that the antibody carries, either on an element of its structure, for example the constituent tyrosine residues, or on an appropriate radical which has been fixed thereto, a radioactive isotope which enables it to be assayed by counting the radioactivity associated therewith.

The expression "enzymatic labeling" means that the antibody is coupled with an enzyme which, when associated with appropriate reagents, allows a quantitative measurement of this specific antibody.

The substrate and reagents are chosen so that the final product of the reaction or reaction sequence caused by the enzyme and involving these substances is:

- either a colored or fluorescent substance which diffuses into the liquid medium surrounding the test sample and which is subjected either to the final spectrophotometric or, respectively, fluorimetric measurement or to a visual evaluation, optionally against a color scale,

- or an insoluble colored substance which deposits on the test sample and which can be subjected either to measurement with a reflection photometer or to a visual evaluation, optionally against a color scale.

When using an antibody which has been rendered fluorescent, the fluorescence associated with the test sample is read off directly on an appropriate apparatus.

When using a radioactive probe, for example iodine 125, the radioactivity associated with the test sample is counted in a gamma counter by any appropriate mode of implementation and e.g. after solubilization of the cells with an alkaline solution (for example sodium hydroxide solution) and recovery of the solution containing the radioactivity by using an absorbent plug.

When using an enzyme on the specific antibody, the appearance of a colored or fluorescent product is obtained by adding a solution containing the enzyme substrate and one or more auxiliary reagents making it possible finally to obtain, as the reaction product, either a colored product soluble in the medium, or an insoluble colored product, or a soluble fluorescent product, as explained above. The light signal originating from the samples treated in this way is then measured with an apparatus appropriate to each case, namely a transmission or reflection photometer or a fluorimeter, respectively. Alternatively, the coloration obtained can also be evaluated visually, optionally with the help of a scale of colored solutions.

If alkaline phosphatase is used as the enzyme, this enzyme is coupled with the specific antibody by the method proposed by Boehringer Mannheim-Biochemica. The preferred substrates for this enzyme are paranitrophenyl phosphate if the final measurement is to be spectrophotometric, or 4-methyl-umbelliferyl phosphate is the measurement is to be fluorimetric, or 5-bromo-4-chloro-3-indolyl phosphate if an insoluble colored reaction product is to be obtained. β-Galactosidase can likewise be used as the enzyme, the preferred substrates being orthonitrophenyl β-D-galactopyranoside or 4-methylumbelliferyl β-D-galactopyranoside.

Preferably, the specific antibodies can be coupled with peroxidase. In this case the coupling process is based on that described by M.B. WILSON and P.K. NAKANE in Immunofluorescence and Related Staining Techniques, W. Knapp, K. Kolubar, G. Wicks ed., Elsevier/North Holland, Amsterdam 1978, pp. 215-224.

The reagents used to reveal the peroxidase conjugated with the specific antibodies contain hydrogen peroxide (enzyme substrate) and an appropriate chromogen, for example orthophenylenediamine or 2,2′-azinobis(3-ethylthiazoline-6-sulfonic) acid [ABTS], to give a colored final reaction product soluble in the medium, or 3,3′-diaminobenzidine, 3-amino-9-ethylcarbazole or 4-chloro-α-naphthol to give an insoluble final reaction product, or parahydroxyphenylpropionic acid to give a fluorescent reaction product soluble in the medium.

Another embodiment of the invention is the use of specific antibodies coupled with acetylcholinesterase.

Acetylcholinesterase is preferably coupled with the antibody by means of a process based on that described in French patent no. 2 550 799 or by a process which briefly comprises preparing fragments of the antibody by a known technique, modifying the enzyme by reaction with an appropriate heterobifunctional agent and, finally, coupling the resulting products. Other known processes for the construction of immunoenzymatic conjugates can also be used in this case.

The enzymatic activity specifically bound to the antigen recognized by the acetylcholinesterase conjugate is preferably revealed by the well-known technique which employs acetylthiocholine as the enzyme substrate and Ellman's reagent (5,5′-dithio-2-nitrobenzoic acid) as the chromogen using any variant suitable for the case in question, for example the one described by Pradelles et al. in Anal. Chem. 1985, 57: 1170-1173.

The chromogens mentioned are used as such or in the form of water-soluble salts.

The method of serological diagnosis of the invention is suitable for use in biology and/or anatomicopathological laboratories. For this purpose, the device proposed for carrying out this method comprises a solid support on or in which a solution containing the bacterium as defined above has been deposited.

According to another feature, the invention further relates to a kit for *in vitro* detection of the bacterium responsible for Whipple's disease. This kit comprises the following components:

- a solution containing the bacterium or an antigen according to the invention; and/or

- a solution containing at least one antibody according to the invention; and/or

- a solution containing at least one antibody specific for a human immunoglobulin which recognizes said ... .

More particularly, the kit comprises:

- a solution containing the bacterium responsible for Whipple's disease, isolated and established as described above, as a positive control;

- a solution containing a labeled specific antibody; and

- optionally a washing solution.

The specific antibody used in the kit of the invention is advantageously labeled with a radioactive probe, an enzyme or a fluorescent agent.

When the specific antibody is labeled with an enzyme, the kit also comprises the enzyme substrate and one or more reagents for visualizing the enzymatic activity.

When the specific antibody is labeled with a fluorescent agent, it is preferred to use fluorescein iso(thio)cyanate.

In one preferred embodiment of the invention, the specific antibody used is an immunoglobulin, particularly a mouse immunoglobulin.

The present invention further relates to the *rpoB* gene of the bacterium *Tropheryma whippelii* according to the present invention. The sequence of the *rpoB* gene was determined by enzymatic amplification and direct automatic sequencing with consensus primers among a large number of other bacteria of different genera and species.

The *rpoB* gene codes for one of the subunits of bacterial RNA polymerase and constitutes a genetic marker enabling specific detection of the bacterium of the species *Tropheryma whippelii*.

More particularly, the present invention relates to a fragment of the *rpoB* gene, characterized in that it has the nucleotide sequence SEQ ID N° 3 in the attached sequence listing.

The present invention therefore further relates to nucleic acid sequences specific for the species *Tropheryma whippelii* whose nucleotide sequence is derived from the *rpoB* gene of said bacterium and especially from the fragment of the *rpoB* gene referred to above.

According to Lazcano et al. [J. Mol. Evol. (1988) 27: 365-376], RNA

polymerases are divided into two groups according to their origin, one consisting of RNA- or DNA-dependent viral RNA polymerases and the other consisting of DNA-dependent RNA polymerases of eukaryotic or prokaryotic origin (archaebacteria and eubacteria). Eubacterial DNA-dependent RNA polymerases are characterized by a simple and conserved multimeric constitution called a "core enzyme", represented by $\alpha\beta\beta'$, or a "holoenzyme", represented by $\alpha\beta\beta'\sigma$ [Yura and Ishihama, Ann. Rev. Genet. (1979) 13: 59-97].

Numerous studies have demonstrated the functional role, within the multimeric enzymatic complex, of the β subunit of eubacterial RNA polymerase. As far as archaebacterial and eukaryotic RNA polymerases are concerned, they have a more complex structure capable of reaching about ten or even about thirty subunits (Pühler et al., Proc. Natl. Acad. Sci. USA (1989) 86: 4569-4573].

The genes which code for the different $\alpha\beta\beta'\sigma$ subunits of DNA-dependent RNA polymerase in eubacteria, namely the *rpoA, rpoB, rpoC* and *rpoD* genes respectively, are classed in different groups comprising genes coding for constituent proteins of the ribosomal subunits or for enzymes involved in the replication and repair of the genome [Yura and Ishihama, Ann. Rev. Genet. (1979) 13: 59-97]. Some authors have shown that the nucleic acid sequences of the *rpoB* and *rpoC* genes can be used to construct phylogenic trees [Rowland et al., Biochem. Soc. Trans. (1992) 21: 40s], making it possible to separate the different branches and sub-branches among the kingdoms of the living.

Before this feature of the invention is explained in greater detail, various terms used in the description and the claims are defined below:

- "nucleic acid extracted from bacteria" is understood as meaning either the whole nucleic acid, or the genomic DNA, or the messenger RNAs, or the DNA obtained from reverse transcription of the messenger RNAs.

- "nucleotide fragment" and "oligonucleotide" are two synonymous terms denoting a concatenation of nucleotide units which is characterized by an information sequence of natural (or possibly modified) nucleic acids capable of hybridizing, like natural nucleic acids, with a complementary or substantially complementary nucleotide fragment under predetermined conditions of strict stringency. The concatenation can contain nucleotide units whose structure differs from that of natural nucleic acids. A nucleotide fragment (or oligonucleotide) can contain e.g. up to 100 nucleotide units. It contains generally at least 10 and

particularly at least 12 nucleotide units and can be obtained from a natural nucleic acid molecule and/or by genetic recombination and/or by chemical synthesis.

- a nucleotide unit is derived from a monomer which can be a natural nucleic acid nucleotide whose constituent elements are a sugar, a phosphate group and a nitrogen base selected from adenine, guanine, uracil, cytosine and thymine; alternatively, the monomer is a nucleotide modified in at least one of the three constituent elements listed above; by way of example, the modification can take place either at the bases, with modified bases such as inosine, 5-methyldeoxycytidine, deoxyuridine, 5-dimethylaminodeoxyuridine or any other modified base capable of hybridization, or at the sugar, for example by replacement of at least one deoxyribose with a polyamide [P.E. Nielsen et al., Science (1991) 254: 1497-1500], or at the phosphate group, for example by replacement with esters selected especially from diphosphates, alkyl- and arylphosphonates and phosphorothioates.

- "information sequence" is understood as meaning any ordered series of nucleotide-type units whose chemical nature and whose order in a reference direction constitute information analogous to that given by the sequence of natural nucleic acids.

- "hybridization" is understood as meaning the process during which, under appropriate conditions, two nucleotide fragments having sufficiently complementary sequences are capable of associating with one another via stable and specific hydrogen bonds to form a double strand. The hybridization conditions are determined by the "stringency", i.e. the rigor of the operating conditions. The greater the stringency, the more specific is the hybridization. The stringency is a function especially of the base composition of a probe/target duplex and by the degree of mismatch between two nucleic acids. The stringency can also be a function of the parameters of the hybridization reaction, such as the concentration and type of ionic species present in the hybridization solution, the nature and concentration of denaturing agents and/or the hybridization temperature. The stringency of the conditions under which a hybridization reaction has to be performed depends especially on the probes used. All these facts are well known and the appropriate conditions can optionally be determined in each case by means of routine experiments. In general, depending on the length of the probes used, the temperature of the hybridization reaction is between about 20 and 65°C,

particularly between 35 and 65°C, in a saline solution with a concentration of about 0.8 to 1 M.

- a "probe" is a nucleotide fragment comprising e.g. from 10 to 100 nucleotide units, especially from 12 to 35 nucleotide units, and possessing a hybridization specificity under given conditions for forming a hybridization complex with a nucleic acid which, in the present case, has a nucleotide sequence included either in a messenger RNA or in a DNA obtained by reverse transcription of said messenger RNA, i.e. a transcription product; a probe can be used for diagnostic purposes (especially capture or detection probes) or for therapeutic purposes.

- a "capture probe" is immobilized or immobilizable on a solid support by any appropriate means, for example by covalent bonding, by adsorption or by direct synthesis on a solid. Examples of supports include microtiter plates and DNA chips.

- a "detection probe" can be labeled with a marker selected e.g. from radioactive isotopes, enzymes, particularly those capable of acting on a chromogenic, fluorigenic or luminescent substrate (especially a peroxidase or an alkaline phosphatase), chromophoric chemicals, chromogenic, fluorigenic or luminescent compounds, nucleotide base analogs, and ligands such as biotin.

- a "species probe" is a probe for identifying the species of a bacterium.

- a "genus probe" is a probe for identifying the genus of a bacterium.

- a "primer" is a probe comprising e.g. from 10 to 100 nucleotide units and possessing a hybridization specificity under given conditions for initiating an enzymatic polymerization, for example in an amplification technique such as PCR, in a sequencing process, in a transcription method, etc.

One subject of the present invention is a single-stranded oligonucleotide selected from oligonucleotides having a sequence of at least 12 consecutive nucleotide units included in one of the sequences SEQ ID N° 4 and SEQ ID N° 5 in the attached sequence listing, and from the oligonucleotides complementary to these oligonucleotides. These oligonucleotides can be oligodeoxyribonucleotides (DNAs) and oligoribonucleotides (RNAs) in which the "T" is replaced with "U".

In particular, an oligonucleotide according to the present invention possesses at least 12 units as described above and at most 50 units. More particularly, an oligonucleotide according to the present invention possesses from

12 to 35 units.

A preferred oligonucleotide has a sequence selected from the sequences SEQ ID N° 4 and 5.

Inosine is capable of pairing with any other base.

The sequences SEQ ID N° 4 and 5 can be prepared by chemical synthesis using the techniques well known to those skilled in the art and described e.g. in the article by Itakura K. et al. [(1984) Annu. Rev. Biochem. 53: 323].

A first application of an oligonucleotide of the invention is its use as a probe for detecting bacteria of the species *Tropheryma whippelii* in a biological sample, said probe comprising a nucleotide sequence of at least 12 consecutive nucleotide units included in one of the sequences SEQ ID N° 4 and SEQ ID N° 5 and their complementary sequences. In the remainder of the description, such a probe of the invention will be called a species probe.

The probes according to the invention can be used for diagnostic purposes in tests for detecting the presence or absence of a target nucleic acid in a sample by any of the known hybridization techniques, especially the techniques called "DOT-BLOT" [Maniatis et al. (1982) Molecular Cloning, Cold Spring Harbor], the DNA transfer techniques called "SOUTHERN BLOTTING" [Southern E.M., J. Mol. Biol. (1975) 98: 503], the RNA transfer techniques called "NORTHERN BLOTTING" or the so-called "sandwich" techniques [Dunn A.R., Hassel J.A. (1977) Cell 12: 23]. In particular, the "sandwich" technique is used with a capture probe and/or a detection probe, said probes being capable of hybridizing with two different regions of the target nucleic acid, and at least one of said probes (generally the detection probe) being capable of hybridizing with a region of the target which is specific for the species, it being understood that the capture probe and the detection probe must have at least partially different nucleotide sequences.

The nucleic acid to be detected (target) can be DNA or RNA (one or other optionally being obtained after amplification by PCR). In the case of the detection of a target of the double-stranded nucleic acid type, it is appropriate to denature the latter before carrying out the detection process. The target nucleic acid can be obtained by extraction from the nucleic acids of a test sample using the known methods. A double-stranded nucleic acid can be denatured by the known methods of chemical, physical or enzymatic denaturing and particularly by heating to an appropriate temperature above 80°C.

To carry out the above-mentioned hybridization techniques and particularly the "sandwich" techniques, one probe of the invention, called a capture probe, is immobilized on a solid support and another probe of the invention, called a detection probe, is labeled with a marker.

Examples of supports and markers are as defined above.

A further subject of the invention is a process for determining the presence or absence of a *Tropheryma whippelii* bacterium in a sample which contains or may contain nucleic acids of at least one such bacterium, said process comprising the steps which consist in bringing said sample into contact with at least one species probe of the invention and then determining, in a manner known per se, the formation or absence of formation of a hybridization complex between said probe and the nucleic acid of the sample.

Examples of how to detect the formation or absence of formation of a hybridization complex between said probe and the nucleic acid include the techniques described above, namely the "DOT-BLOT", "SOUTHERN BLOTTING" and "sandwich" techniques.

In one particular mode of carrying out this process for determining the presence or absence of the species *Tropheryma whippelii*, several species probes of the invention are used, it being understood that said probes are capable of hybridizing with non-overlapping regions of a nucleic acid corresponding to the *rpoB* gene of *Tropheryma whippelii*.

Advantageously, one species probe is immobilized on a solid support and another species probe is labeled with a marker.

Another application of an oligonucleotide of the invention is its use as a nucleotide primer comprising a single-stranded oligonucleotide selected from oligonucleotides having a sequence of at least 12 nucleotide units included in one of the sequences SEQ ID N° 4 and 5, which primer can be used in the synthesis of a nucleic acid in the presence of a polymerase by a process known per se, especially in amplification methods using such a synthesis in the presence of a polymerase (PCR, RT-PCR, etc.). In particular, a primer of the invention can be used for the specific reverse transcription of a messenger RNA sequence of *Tropheryma whippelii* to give a corresponding complementary DNA sequence. Such a reverse transcription can constitute the first stage of the RT-PCR technique, the next stage being the amplification of the resulting complementary DNA by

PCR. The primers of the invention can also be used for specific amplification of the total sequence of the DNA of the *rpoB* gene of *Tropheryma whippelii* by the polymerase chain reaction.

In one particular case, said primer comprising an oligonucleotide of the invention also comprises the sense or antisense sequence of a promoter recognized by an RNA polymerase (e.g. T7, T3 or SP6 promoter [Studier F.W., B.A. Moffatt (1986) J. Mol. Biol. 189: 113]: such primers can be used in nucleic acid amplification processes involving a transcription step, for example the NASBA or 3SR techniques [Van Gemen B. et al., Abstract MA 1091, 7th International Conference on AIDS (1991) Florence, Italy].

A further subject of the invention is a nucleotide primer comprising a single-stranded oligonucleotide selected from oligonucleotides having a sequence of at least 12 consecutive nucleotide units included in one of the sequences SEQ ID N° 4 and SEQ ID N° 5, which primer can be used for the total or partial sequencing of the *rpoB* gene of any strain of *Tropheryma whippelii*. In particular, the nucleotide primer can be used for the sequencing of an amplified nucleic acid, said sequencing giving the total or partial sequence of the *rpoB* gene by a process known per se, i.e. absorptive polymerization using dideoxynucleotides [Sanger F., Coulson A.R. (1975) J. Mol. Biol. 94: 441] or multiple hybridizations using DNA chips.

Preferably, in a use as a primer or for the sequencing of the *rpoB* genes, the sequences SEQ ID N° 4 and 5 are used.

Finally, a last subject of the invention is a gene therapy probe for treating infections caused by a strain of *Tropheryma whippelii*, said probe comprising an oligonucleotide as defined above. This gene therapy probe, which is capable of hybridizing with the messenger RNA and/or the genomic DNA of said bacteria, can block the phenomena of translation and/or transcription and/or replication.

The principle of gene therapy methods is known and is based in particular on the use of a probe corresponding to an antisense strand: the formation of a hybrid between the probe and the sense strand is capable of perturbing at least one of the steps involving decryption of the genetic information. Gene therapy probes can therefore be used as antibacterial drugs for combating infections caused by spirochetes.

The invention will be understood more clearly with the aid of the following

account divided into Examples. Said Examples relate to experiments performed for the purpose of putting the invention into effect and are given purely by way of illustration.

Figures 1 to 4 are photographs of electrophoresis gel.

Figure 1 shows the SDS-PAGE protein profile of *Tropheryma whippelii*.

Figure 2 shows the antigen profile of *Tropheryma whippelii* obtained by Western blotting.

Lane 1 = immunized mouse serum.

Lane 2 = immunized rabbit serum.

Lane 3 = patient serum (IgG + IgM).

Lane 4 = monoclonal antibody 1.

Lane 5 = monoclonal antibody 2.

Lane 6 = monoclonal antibody 3.

Lane 7 = monoclonal antibody 4.

Figure 3 shows a Western blot performed on the strain TWIST no. I-2202 with the serum of a patient suffering from Whipple's disease, with IgM detection.

Figure 4 shows the visualization of the product resulting from the amplification of the *rpoB* gene of *Tropheryma whippelii* with primers SEQ ID N° 1 and 2 after staining with ethidium bromide.

Lane 1: *Tropheryma whippelii*

Lane 2: *Nocardia otitidiscaviarum*

Lane 3: *Mycobacterium tuberculosis*

Lane 4: *Staphylococcus epidermidis*

Lane 5: *Corynebacterium amycolatum*

Lane 6: *Mycobacterium avium*

Lane 7: *Escherichia coli*

Lane 8: H_2O

Lane 9: H_2O

Example 1: Primary isolation of the bacterium

The primary isolation was effected by the centrifugation technique on bijoux tubes inoculated with human fibroblasts of the HEL line available from the ATCC. The HEL cells are cultivated on MEM (Gibco) supplemented with 10% of fetal calf serum (Gibco) and with 2 mM L-glutamine (Gibco). The bijoux tubes (Sterilin - Feltham - England, 3.7 ml), containing a carrier slide 12 mm in diameter,

are inoculated with 1 ml of culture medium containing about 50,000 cells and incubated at 37°C for 3 days under 5% of CO_2 to give a mat of confluent cells. The heart valve studied was ground in MEM and the suspension was used to inoculate the 3 bijoux tubes. These tubes were then centrifuged at 700 g for 1 hour at 22°C. The supernatant was then withdrawn and the mats were washed twice with sterile PBS and then incubated with 1 ml of medium at 37°C under 5% of CO_2. The cultures were monitored by the cytocentrifugation of 100 µl of the supernatant in the bijoux tubes and Gimenez staining. This procedure was repeated after 10, 20 and 30 days. After 30 days the supernatant and the cellular mat in the bijoux tubes were harvested and subcultured on a confluent cellular mat in a 25 cm^2 culture dish (dish I) containing 15 ml of culture medium and incubated at 37°C under 5% of CO_2. Every week for the next 6 weeks (D72), the cellular mat was examined under an inverted microscope to look for a cytopathogenic effect and the culture medium was replaced with fresh medium. Before the change of medium, 200 µl of supernatant were used to carry out a cytocentrifugation and Gimenez staining.

No cytopathogenic effect was detected before day 65. On day 72, small, dark and irregular inclusions could be detected in the HEL cells on examination of the cellular mat by inverted microscopy. After cytocentrifugation of the supernatant in dish I and Gimenez staining, several fine bacilli were detected, the majority of them being located inside the cells, where they appear smaller than the extracellular bacilli. Nevertheless, the majority were poorly stained, if at all, by Gimenez staining and appeared pale blue. Numerous bacilli were also detected after Gram staining. The majority appeared Gram-positive, but several were only partially violet or appeared Gram-negative. These bacilli are not acid-fast after Ziehl staining. After staining with PAS, the PAS-positive bacilli appeared more numerous than after the previous stainings. The majority of the long fine bacilli are observable outside the cells. The HEL cells appear filled with PAS-positive conglomerates and short fine PAS-positive bacilli.

Example 2: Propagation of the isolate

The entire propagation procedure was carried out on HEL cells cultivated on MEM medium supplemented with 10% of fetal calf serum and with 2 mM L-glutamine, and incubated at 37°C under 5% of CO_2. On day 75, 3 ml of supernatant in dish I were used to inoculate 10 bijoux tubes by the method

described above and 2 ml of supernatant were used to inoculate a confluent cellular mat in a 25 cm^2 culture dish (dish A) containing 15 ml of medium. The cells in dish I and the remainder of the supernatant were harvested to give 10 ml of suspension. This suspension was then divided into five 2 ml aliquots. One of the aliquots was frozen in liquid nitrogen. Another aliquot was inoculated onto a confluent cellular mat in a 25 cm^2 culture dish (dish B) containing 15 ml of medium. The cells of another aliquot were lyzed by means of 4 freeze-thaw cycles using liquid nitrogen and warm water (55°C) and then inoculated onto a confluent cellular mat in a 25 cm^2 culture dish (dish C) containing 15 ml of medium. Another aliquot was inoculated into a 25 cm^2 culture dish (dish D) containing 15 ml of medium but no cellular mat. On day 85 the medium in all these dishes and bijoux tubes was replaced with fresh medium. The cells were harvested and inoculated into a 75 cm^2 culture dish (dish D2) containing 30 ml of medium. Before the change of medium, 200 µl of supernatant were used to carry out a cytocentrifugation and staining with PAS and the remainder of the supernatant was frozen to be used as an antigen for serology. On days 95 and 105 the medium in all the dishes and bijoux tubes was changed as described above. Small portions of cellular mat were scraped in order to prepare cellular smears for staining with PAS. The efficiency of propagation of the strain was evaluated semiquantitatively. The presence of PAS-positive bacilli was evaluated microscopically as follows with a magnification of 1000X: 0, absent; +, present but difficult to find; ++, easy to find but not present in all fields; +++, present in all fields. These evaluations were performed blind.

All the propagation methods proved efficient since they all enabled the isolate to be found after 30 days of subculture (Table 1). The semiquantitative evaluation made it possible to observe that the most efficient procedures are subculture in a bijoux tube, the subculture of supernatant (dish A) and the subculture of cells (dishes D, D2).

Table 1

		Bijoux tube	Dish A	Dish B	Dish C	Dish D	Dish D2
Day 10	Supernatant	+	-	+	-	+	NP
Day 20	Supernatant	+	-	+	+	NP	-

	Cellular smear	NP	+	+	+	NP	-
Day 30	Supernatant	+++	+++	++	+	NP	++
	Cellular smear	NP	++	+	+	NP	++

NP: not performed

Example 3: Detection by immunofluorescence

Intracellular bacteria were detected directly in a bijoux tube by immunofluorescence on day 105. After fixing with acetone, the tube was rinsed twice with PBS. 100 µl of the patient's serum, diluted to 1:50 with PBS containing 3% of skimmed milk powder, were added and the tube was incubated in a humid chamber at 37°C for 30 min. After 3 rinses with PBS, the tube was incubated for 30 min at 37°C with 100 µl of anti-human goat immunoglobulin labeled with fluorescein isothiocyanate (Fluoline H, BioMérieux, Marcy l'Etoile, France), diluted to 1:200 with PBS supplemented with 0.2% of Evans blue. After 3 rinses with PBS, the slide was mounted (cells facing downwards) in buffered glycerol (pH 8) and examined with a magnification of 400X using a Zeiss fluorescence microscope and a confocal microscope (LEICA DMIRBE) equipped with a 100X (NA. 1.4) immersion objective.

Examination by immunofluorescence shows that the staining with PAS and the other stainings underevaluate the cellular infection. On the slide, all the cells are filled with the bacterial antigen after 30 days of subculture. The study by confocal microscopy confirms the intracellular location of the bacterium. Several bacteria are detected in isolation in the form of fine bacilli resembling those observed after PAS staining. Nevertheless, most of the immunopositive material corresponds to larger inclusions where it is impossible to individualize bacteria. No immunopositive material is detected in the nucleus of the cells.

Example 4: Electron microscopy

On day 105, 300 µl of a solution containing the cells harvested from dish D2 were prepared for study by electron microscopy. The cells were fixed in a 2.5% solution of glutaraldehyde in 0.1 M cacodylate buffer containing 0.1 M sucrose, for 1 h at 4°C. The cells were rinsed overnight in the same buffer and then fixed in osmium tetroxide in 0.1 M cacodylate buffer for 1 h at room temperature. Dehydration was effected by successive rinses in ethanol solutions of increasing concentration. The cells were then included in blocks of Epon 812. Thin sections were then cut from the blocks with an LKB Ultratome III microtome and

subsequently stained with a saturated solution of uranyl acetate in methanol and an aqueous solution of lead citrate before being examined under a Jeol JEM 1200 EX electron microscope.

The electron microscope study confirms that the PAS-positive inclusions and the immunopositive material correspond to intact bacteria or bacteria undergoing degradation. The cytoplasmic membrane of these bacteria is composed of two layers dense to electrons. The thin bacterial wall is covered in places with an external pseudo-membrane, which gives a trilamellar appearance. Bacteria in the process of division are observed.

Example 5: Production of mouse polyclonal antibodies against the bacterium responsible for Whipple's disease

A mouse of the Balb C strain was injected intraperitoneally with 0.5 ml of supernatant containing 10^4 bacteria responsible for Whipple's disease. The mouse was then reinjected 1, 2 and 3 weeks later with 0.5 ml of the same suspension. The mouse was bled 1 week after this last inoculation. The serum was tested on the one hand against a culture of the bacterium responsible for Whipple's disease and on the other hand against the valve of a patient suffering from Whipple's disease, once by immunofluorescence and once by the immunoperoxidase method. The revealing antibodies were anti-mouse antibodies labeled with fluorescein or with immunoperoxidase (supplied by Immunotech).

The bacteria could be visualized inside the cells. The present patent application therefore further relates to direct detection of the bacterium responsible for Whipple's disease in biopsies and various swabs, for example a heart valve, a digestive biopsy or a biopsy of any other organ suspected of being infected with the bacterium responsible for Whipple's disease.

Example 6: Production and use of monoclonal antibodies to products against *Tropheryma whippelii*, Twist-Marseille strain, and determination of antigens

Equipment and methods

Strain of *Tropheryma whippelii*. The strain of *Tropheryma whippelii* used to produce and screen the hybridomas and test the specificity of the monoclonal antibodies (Mabs) is the TWIST-Marseille strain deposited in the CNCM under no. I-2202. *Tropheryma whippelii* was cultivated on embryonic human fibroblasts (HEL) under the culture conditions described above. On day 75 the infected cells

from one flask were withdrawn ... centrifuged for 10 minutes at 4000 g. The centrifugation residue was then resuspended in 5 ml of PBS. 0.5 ml of this suspension was inoculated into each mouse. The bacteria were also purified under a Renografin gradient and resuspended in deionized water for SDS-PAGE or in PBS for microimmunofluorescence (MIF).

Production of monoclonal antibodies (Mabs). Six-week-old female BALB/C mice were inoculated three times at 7-day intervals by the intraperitoneal injection of 0.5 ml of a suspension of *Tropheryma whippelii* TWIST-Marseille no. I-2202 in PBS. One week after the last of the 3 injections, the mice received an i.v. booster dose of 0.1 ml of a suspension of *Tropheryma whippelii* in PBS. Three days later, the spleen of the immunized mice was removed and the splenocytes were fused with SP2/0-Ag14 myeloma cells (10:1) using 50% polyethylene glycol (molecular weight: 1300-1600; Sigma Chemical Co., St Louis, Mo). The fused cells were cultivated in a hybridoma culture medium (Seromed, Berlin, Germany) supplemented with 20% of fetal calf serum (Gibco BRL) and with hypoxanthine aminopterin-thymidine (Sigma Chemical Co., St Louis, Mo), at 37°C in a humid atmosphere enriched with 5% of CO_2.

The presence of anti-*T. whippelii* antibodies in the supernatant was detected by MIF. The positive hybridomas were subcultivated for the production of ascites. The isotypes of the Mabs were determined with the aid of the Immuno Type Mouse Monoclonal Antibody Isotyping Kit (SIGMA) containing IgM, IgA, IgG1, IgG2a, IgG2b and IgG3 mouse antisera (Sigma). The specificity of the Mabs was tested by Western blotting. One week after intraperitoneal injection of the mice with 0.5 ml of pristane (2,6,10,14-tetramethylpentadecane; Sigma), the ascites antibodies were produced by the intraperitoneal injection of a suspension of 3×10^6 hybridomas in 0.5 ml of PBS.

Microimmunofluorescence (MIF). MIF was used to screen the hybridomas and to determine the specificity of the Mabs. The antigens, consisting of cultures of *Tropheryma whippelii*, were deposited on 24-well slides using a quill. After fixing with methanol for 10 minutes at room temperature, the Mabs were deposited and incubated in a humid chamber at 37°C for 30 minutes. The slides were subsequently rinsed for 2 x 5 minutes in PBS and then in distilled water, dried in air and then incubated for 30 minutes at 37°C with anti-mouse IgM and anti-mouse IgG goat antibodies conjugated with fluorescein, diluted to 1/200 in PBS

containing 0.2% of Evans blue (BioMérieux, Marcy l'Etoile, France). After rinsing, the slides were mounted using Fluorep (BioMérieux) and then read at a magnification of 400X under a fluorescence microscope (Axioskop 20; Carl Zeiss, Göttingen, Germany). The sera of the immunized mice were used as a positive control and the sera of the healthy mice as a negative control.

To detect the anti-*Tropheryma whippelii* antibodies in the serum of patients suffering from Whipple's disease, MIF was performed on Labtech slides [Raoult D. et al., N. Engl. J. Med., 2000]. The sera were diluted to 1:50, 1:100, 1:200, 1:400 and 1:800.

SDS-PAGE and Western blotting. SDS-PAGE and Western blotting were performed by Laemmli's method modified by the use of a 12% polyacrylamide separating gel and a 5% transfer gel. A suspension of *Tropheryma whippelii* containing 4 mg/ml of protein in buffer (0.0625 M Tris hydrochloride [pH 8.0], 2% of SDS, 5% of 2-mercaptoethanol, 10% of glycerol, 0.02% of bromophenol blue) was heated at 100°C for 5 minutes. The dissolved antigens were separated by gel electrophoresis with a constant intensity of 8 to 10 mA for 3 to 4 hours in an electrophoresis cell (Mini Protein II: Bio Rad, Richmond, Calif.) (migration buffer: 25 mM Tris, 192 mM glycine, 0.1% of SDS). The size of the proteins was determined by comparison with a peptide weight marker (Low Range; Bio Rad). The antigens separated in this way were transferred to a nitrocellulose membrane (0.45 µm pores) using a transfer buffer (2.5 mM Tris, 192 mM glycine, 20% of methanol) under a current of 50 V for 1 hour at +4°C in a Western blotting cell (Mini Trans-Blot; Bio Rad). After transfer, the nitrocellulose membranes were incubated overnight with 5% skimmed milk solution to block the non-specific binding sites. The membranes were subsequently washed 3 times in PBS and then dried in air. They were then incubated with the hybridoma supernatant diluted to 1:4 or with the patients' serum diluted to 1:100 in PBS supplemented with 3% of milk, at room temperature for 1 hour, and were then washed as described above. The membranes were subsequently incubated at room temperature for 1 hour with an F(ab')2 fragment of anti-mouse IgG goat antibody conjugated with peroxidase (Heavy and light chains: AffiniPure; Jackson ImmunoResearch), diluted to 1:500 in PBS supplemented with 3% of skimmed milk, and were then washed in PBS. The presence of specific antibodies was revealed by the presence of peroxidase activity using the substrate 4-chloro-1-naphthol.

Blind tests on the Mabs. The specificity of the monoclonal antibodies was evaluated blind by MIF on 19 bacteria: 12 species of *Bartonella*, namely 5 *B. quintana, B. henselae Marseille, B. henselae* Houston, *B. vinsonii* Baker, *B. elizabethae, B. grahamii, B. doshiae* and *B. taylorii, Coxiella burnetii* Nine Mile and a variety of 6 strains isolated in our laboratory from clinical samples, namely *Listeria monocytogenes, Staphylococcus aureus, Streptococcus bovis, Mycobacterium avium, Corynebacterium* ANF group and *Actinomyces mayerii*. After suspension in PBS and deposition on slides containing wells, the reactivity of the Mabs with the bacteria was estimated by MIF as described above with ascitic fluid diluted to 1:100.

Patients. 15 patients were tested: 8 patients with Whipple's disease located solely in the digestive system, which had been diagnosed by histology and/or the amplification of *T. whippelii* by PCR in digestive biopsies; and 7 patients suffering from endocarditis caused by *T. whippelii* ... by PCR in valve biopsies.

Mice and rabbits. 5 mice and 1 rabbit were inoculated with a suspension of *T. whippelii* in order to determine what antigens would be recognized by the antibody response.

Results

SDS-PAGE profiles (Fig. 1). SDS-PAGE of *T. whippelii* showed more particularly 7 main bands at 10, 20, 80, 120, 150, 170 and 200 kDa.

Production of the Mabs. The supernatant of 4 hybridomas was tested by MIF. Four hybridomas proved specific for *T. whippelii*. One hybridoma was deposited in the CNCM of the Institut Pasteur, 25 rue du Docteur Roux, PARIS 75724, under no. I-2411 and under the identification reference TW 17G2.

Serological tests performed on the patients. 13 of the 15 patients (86.7%) gave a positive serology test with an IgM level of ≥1:100. The antigen profile observed in Western blotting showed a reactivity with several bands, including a major band at 200 kDa for IgG (Figure 2). Several protein profiles are identified for IgM, including a reactivity against proteins of 100, 60, 50 and 35 kDa (Figure 3).

Serological tests performed on the immunized mice and rabbit. The immunized mice and rabbit gave a positive serology test with an IgM level of >1:100. The antigen profile observed in Western blotting showed a different reactivity between mouse and rabbit which differed from that observed in the

patients. On the other hand, a major reactivity was observed with a band at 200 kDa.

Characterization of the Mabs (Fig. 2). The 4 Mabs exhibited a specific reactivity for an antigen of 200 kDa, which had already been demonstrated on the Western blots of the patients, mice and rabbit. They were all identified as IgMs. The recognized antigen was destroyed by the action of proteinase K, showing that it was a protein.

Blind tests on the Mabs. The ascites of the 4 hybridomas reacted only with *T. whippelii*.

Example 7: Sequence of the *rpoB* gene of *Tropheryma whippelii*

The sequence of the *rpoB* gene of *Tropheryma whippelii* was determined by enzymatic amplification and direct automatic sequencing using consensus primers.

The sequences of the consensus primers used were as follows:

SEQ ID N° 1 = 5′-TIA TGG GII CIA AIA TGC A-3′

SEQ ID N° 2 = 5′-GCC CAI CAT TCC ATI TCI CC-3′ (I = inosine)

DNA extracted from the strain *Tropheryma whippelii* Twist-Marseille CNCM I-2202 by mechanical and chemical lysis (Fast-Prep Bio 101) was incorporated under the following experimental conditions: 35 amplification cycles, each cycle comprising a denaturation of the DNA at 94°C for 30 sec., an initial hybridization of the primers at 50°C for 30 sec. including a ramping of -0.1°C per cycle, and then an elongation at 72°C for 60 sec.

The sequences SEQ ID N° 1 and SEQ ID N° 2 were determined by alignment of the peptide sequences deposited in GenBank under the following accession numbers for the following bacteria: *Bacillus subtilis*, L43593, *Bartonella henselae*, AF171070, *Borrelia burgdorferi*, AE001144, *Buchnera aphidicola*, Z11913, *Chlamydia pneumoniae*, AE001593, *Chlamydia trachomatis*, AE001304, *Coxiella burnetti*, U86688, *Escherichia coli*, U76222, *Haemophilus influenzae*, U32733, *Helicobacter pylori*, E000625, *Legionella pneumophila*, AF087812, *Mycobacterium leprae*, Z14314, *Mycobacterium smegmatis*, U24494, *Mycobacterium tuberculosis*, L27989, *Mycoplasma gallisepticum*, L38402, *Mycoplasma genitalium*, U39715, *Mycoplasma pneumoniae*, AE000030, *Neisseria meningitidis*, Z54353, *Pseudomonas putida*, X15849, *Rickettsia prowazekii*, AF034531, *Rickettsia typhi*, P77941, *Salmonella enterica typhimurium*, X04642, *Spiroplasma citri*, U25815, *Staphylococcus aureus*, U970062, *Synechocystis spp.*, D90905,

Thermotoga maritima, X72695, *Treponema pallidum,* AE001205, and *Human Granulocytic Ehrlichiosis agent*, AF237414.

The sequences SEQ ID N° 1 and 2 were chosen by selecting the sequences which were the most conserved, on the assumption that they were also present in *Tropheryma whippelii*.

The partial sequence of the *rpoB* gene of *Tropheryma whippelii* (GenBank accession number AF243072) which corresponds to SEQ ID N° 3, obtained with the aid of the primers SEQ ID N° 1 and N° 2, is as follows:

BASE 157 a 117 c 176 g 150 t 12 others

Example 8: Specific detection of the *rpoB* gene of *Tropheryma whippelii*

The following sequences specific for *Tropheryma whippelii* were selected from the fragment SEQ ID N° 3:

SEQ ID N° 4: 5'-GCA TTG TGG GGG ATG TTT-3'

SEQ ID N° 5: 5'-TTG GGG TCA CCT TAC CAA-3'

They were chosen as being specific for *Tropheryma whippelii* by comparison with the known sequences of the *rpoB* gene of the 28 above-mentioned bacteria listed in GenBank.

Example 9: Specific amplification of the *rpoB* gene of *Tropheryma whippelii*

The *rpoB* gene of *Tropheryma whippelii* was amplified by the PCR technique using 35 amplification cycles each comprising a denaturation phase at 94°C for 30 seconds, a hybridization phase for the primers SEQ ID N° 4 and 5 at 52°C for 30 seconds and an elongation phase at 72°C for 90 seconds. The amplification product is visualized after staining with ethidium bromide (Figure 4).

The control bacteria, namely *Mycobacterium avium, Mycobacterium tuberculosis, Nocardia otitidiscaviarum, Escherichia coli, Staphylococcus eperdimilis* and *Corynebacterium amycolatum*, were not detected, demonstrating the specificity of the primers tested.

The *rpoB* sequences of these control species deposited in GenBank under the following accession numbers: *Mycobacterium avium* ATCC 25291, AF060336, *Mycobacterium tuberculosis,* U12205, and *Escherichia coli* K-12, U77436, were chosen on account of their genetic similarity to *Tropheryma whippelii* or on account of their occurrence as a possible contaminant in the clinical swabs submitted for the detection of *Tropheryma whippelii.*

CLAIMS (modified in response to EPO notification of 19/06/2001)

1. Bacterium *Tropheryma whippelii* responsible for Whipple's disease, isolated and established in culture.

2. Bacterium according to claim 1 obtained from a culture of human fibroblasts after at least 2 months of incubation in a culture medium based on MEM.

3. Bacterium according to claim 1 or 2, characterized in that it is deposited in the CNCM of the Institut Pasteur under the number I-2202.

4. Antigen of a bacterium according to one of claims 1 to 3.

5. Antigen according to claim 4, characterized in that it is a protein selected from those with molecular weights of about 35, 50, 60, 100 and 200 kD determined in Figures 2 and 3 by polyacrylamide gel electrophoresis using the Western blotting technique.

6. Specific antibody directed against the bacterium or an antigen of the bacterium according to one of claims 1 to 5.

7. Antibody according to claim 6, characterized in that it is a polyclonal antibody of animal origin, preferably a mouse immunoglobulin.

8. Antibody according to claim 6, characterized in that it is a monoclonal antibody.

9. Antibody according to claim 8, characterized in that it is a monoclonal antibody produced by a hybridoma deposited in the CNCM of the Institut Pasteur under the registration number I-2411.

10. Antigen according to claim 5, characterized in that it is a protein of 200 kD which reacts with an antibody according to claim 9.

11. Use of a bacterium according to any one of claims 1 to 3 or an antigen according to claim 4, 5 or 10 for the *in vitro* diagnosis of diseases associated with infections caused by the bacterium *Tropheryma whippelii*.

12. Use of an antibody according to one of claims 6 to 9 for *in vitro* diagnosis of the disease associated with infections caused by *Tropheryma whippelii* bacteria.

13. Method for the *in vitro* serological diagnosis of Whipple's disease, comprising the steps which consist essentially in detecting an immunological reaction between an antibody specific for the bacterium according to one of claims 6 to 9 and an antigen of said bacterium according to one of claims 4, 5 and 10.

14. Method for the *in vitro* serological diagnosis of Whipple's disease, comprising the step which consists essentially in detecting an immunological reaction between an antibody specific for a human immunoglobulin which recognizes said bacterium according to one of claims 1 to 3 and a said human immunoglobulin which recognizes said bacterium according to claims 1 to 5.

15. Method of serological diagnosis according to claim 14 comprising the following steps:

- depositing a solution containing the bacterium as defined in claims 1 to 3, in or on a solid support;

- introducing the test serum or biological fluid into or onto said support;

- introducing a solution of a labeled antibody specific for a human immunoglobulin which recognizes said bacterium, into or onto the support;

- observing an incubation period;

- rinsing the solid support; and

- detecting said immunological reaction.

16. Kit for the *in vitro* detection of Whipple's disease by the method of one of claims 13 to 15, essentially comprising the following components:

- a solution containing the bacterium or an antigen as defined in claims 1 to 5 and 10; and/or

- a solution containing at least one antibody according to one of claims 6 to 9; and/or

- a solution containing at least one antibody specific for a human immunoglobulin which recognizes said bacterium according to claims 1 to 3.

17. Kit according to claim 16, characterized in that it comprises at least one labeled specific antibody.

18. Fragment of the *rpoB* gene of the bacterium *Tropheryma whippelii* according to one of claims 1 to 3, characterized in that it comprises the nucleotide sequence SEQ ID N° 3.

19. Oligonucleotide comprising a sequence specific for the *rpoB* gene of the bacterium *Tropheryma whippelii* according to one of claims 1 to 3, said specific sequence comprising at least 12 consecutive nucleotide units included in the sequence SEQ ID N° 3.

20. Single-stranded oligonucleotide according to claim 19 selected from oligonucleotides having a sequence of at least 12 consecutive nucleotide units included in one of the sequences to SEQ ID N° 4 and 5, and from the oligonucleotides complementary to these oligonucleotides.

21. Oligonucleotide according to claim 19 or 20, characterized in that it consists of the sequences SEQ ID N° 4 and 5.

22. Probe for detecting *Tropheryma whippelii* bacteria in a biological sample, characterized in that it comprises a sequence according to claim 18 or an oligonucleotide according to one of claims 19 to 21.

23. Process for determining the presence or absence of a *Tropheryma whippelii* bacterium in a sample which contains or may contain nucleic acids of at least one such bacterium, characterized in that said sample is brought into contact with at least one probe ... claim 22 and the formation or absence of formation of a hybridization complex between said probe and the nucleic acid of the sample is then determined.

24. Nucleotide primer which can be used for synthesizing the *rpoB* gene of *Tropheryma whippelii* in the presence of a polymerase, characterized in that it comprises an oligonucleotide according to claims 19 to 21, preferably an oligonucleotide comprising one of the sequences SEQ ID N° 4 and SEQ ID N° 5.

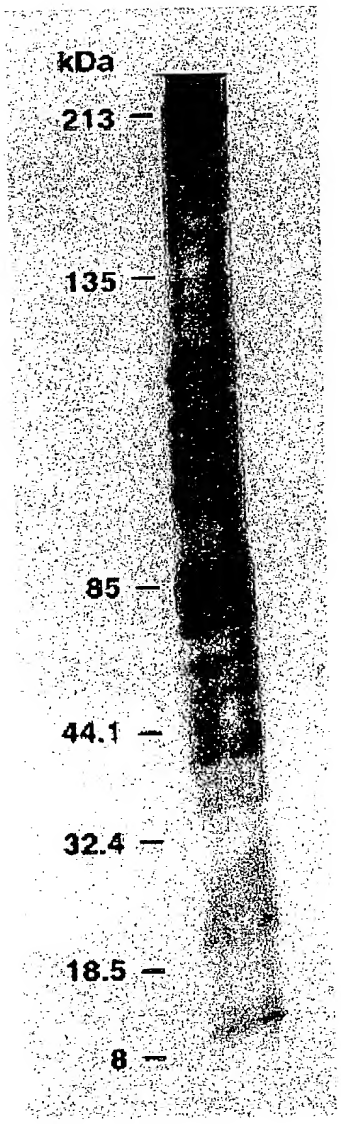

FIG.1

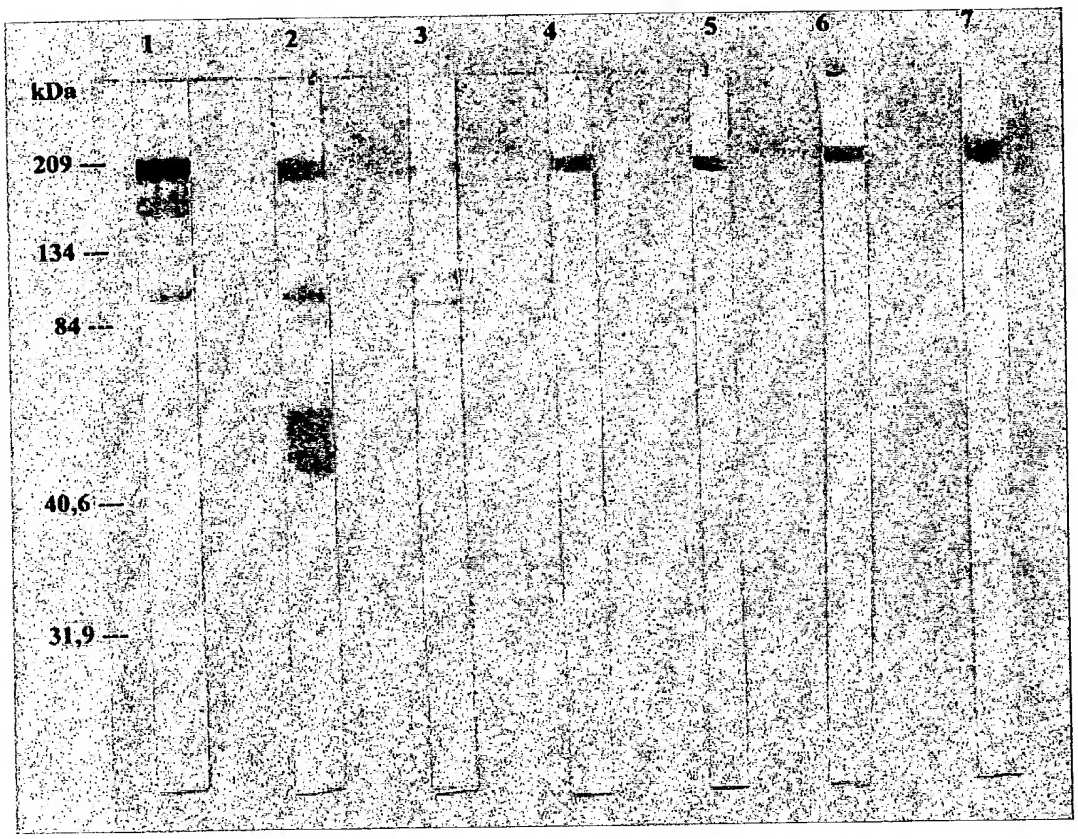

FIG.2

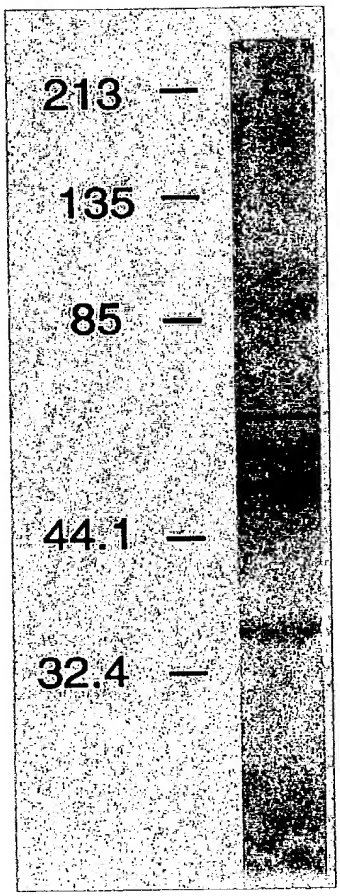

FIG.3

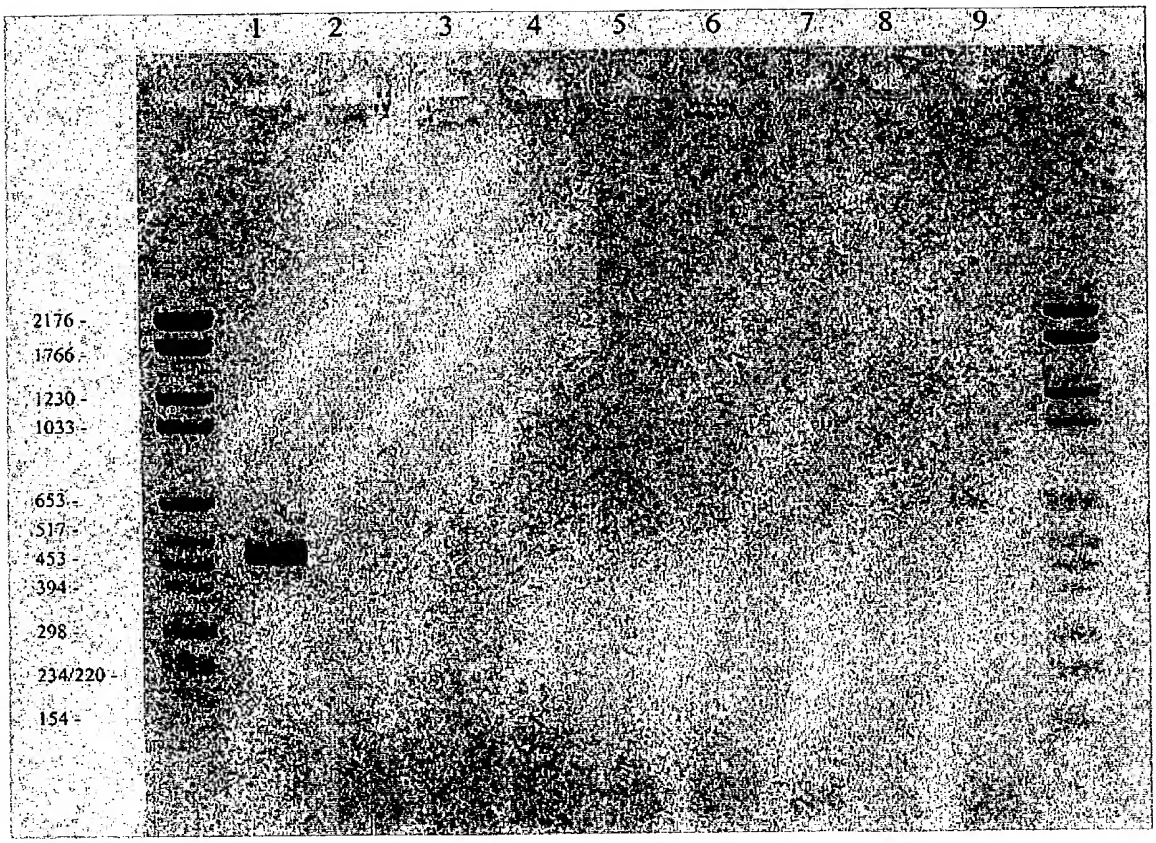

FIG.4

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Declaration and Power of Attorney for Patent Application

Déclaration et Pouvoirs pour Demande de Brevet

French Language Declaration

En tant que l'inventeur nommé ci-après, je déclare par le présent acte que:

As a below named inventor, I hereby declare that:

Mon domicile, mon adresse postale et ma nationalité sont ceux figurant ci-dessous à côté de mon nom.

My residence, post office address and citizenship are as stated next to my name.

Je crois être le premier inventeur original et unique (si un seul nom est mentionné ci-dessous), ou l'un des premiers co-inventeurs originaux (si plusieurs noms sont mentionnés ci-dessous) de l'objet revendiqué, pour lequel une demande de brevet a été déposée concernant l'invention intitulée

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Diagnosis of Whipple's disease

et dont la description est fournie ci-joint à moins que la case suivante n'ait été cochée:

the specification of which is attached hereto unless the following box is checked:

☐ a été déposée le 24/03/2000 sous le numéro de demande des Etats-Unis ou le numéro de demande international PCT PCT/FR00/00754 et modifiée le _____ (le cas échéant).

☐ was filed on _____ as United States Application Number or PCT International Application Number _____ and was amended on _____ (if applicable).

Je déclare par le présent acte avoir passé en revue et compris le contenu de la description ci-dessus, revendications comprises, telles que modifiées par toute modification dont il aura été fait référence ci-dessus.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

Je reconnais devoir divulguer toute information pertinente à la brevetabilité, comme défini dans le Titre 37, § 1.56 du Code fédéral des réglementations.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

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Je revendique par le présent acte avoir la priorité étrangère, en vertu du Titre 35, § 119(a)-(d) ou § 365(b) du Code des Etats-Unis, sur toute demande étrangère de brevet ou certificat d'inventeur ou, en vertu du Titre 35, § 365(a) du même Code, sur toute demande internationale PCT désignant au moins un pays autre que les Etats-Unis et figurant ci-dessous et, en cochant la case, j'ai aussi indiqué ci-dessous toute demande étrangère de brevet, tout certificat d'inventeur ou toute demande internationale PCT ayant une date de dépôt précédant celle de la demande à propos de laquelle une priorité est revendiquée.

I hereby claim foreign priority under Title 35, United States Code, § 119(a)-(d) or § 365 (b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below, and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior foreign application(s) Demande(s) de brevet antérieure(s)			Priority Claimed Droit de priorité revendiqué
FR99/06679	FRANCE	21/05/99	X
(Number) (Numéro)	(Country) (Pays)	(Day/Month/Year Filed) (Jour/Mois/Année de dépôt)	
FR99/03989	FRANCE	26/03/99	X
(Number) (Numéro)	(Country) (Pays)	(Day/Month/Year Filed) (Jour/Mois/Année de dépôt)	

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, § 119(e) du Code des Etats-Unis, de toute demande de brevet provisoire effectuée aux Etats-Unis et figurant ci-dessous.

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(Application No.) (N° de demande)	(Filing Date) (Date de dépôt)
(Application No.) (N° de demande)	(Filing Date) (Date de dépôt)

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I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

(Application No.) (N° de demande)	(Filing Date) (Date de dépôt)	(Status) (patented, pending, abandoned) (Statut) (breveté, en cours d'examen, abandonné)
(Application No.) (N° de demande)	(Filing Date) (Date de dépôt)	(Status) (patented, pending, abandoned) (Statut) (breveté, en cours d'examen, abandonné)

Je déclare par le présent acte que toute déclaration ci-incluse est, à ma connaissance, véridique et que toute déclaration formulée à partir de renseignements ou de suppositions est tenue pour véridique; et de plus, que toutes ces déclarations ont été formulées en sachant que toute fausse déclaration volontaire ou son équivalent est passible d'une amende ou d'une incarcération, ou des deux, en vertu de la Section 1001 du Titre 18 du Code des Etats-Unis, et que de telles déclarations volontairement fausses risquent de compromettre la validité de la demande de brevet ou du brevet délivré à partir de celle-ci.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: *(list name and registration number)*

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(name and telephone number)

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Nom complet du second co-inventeur, le cas échéant LA SCOLA Bernard	Full name of second joint inventor, if any
Signature du second inventeur Date 24/9/01 LA SCOLA Bernard	Second Inventor's signature Date
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1-00

2-00

(Fournir les mêmes renseignements et la signature de tout co-inventeur supplémentaire.)

(Supply similar information and signature for third and subsequent joint inventors.)

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POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: *(list name and registration number)*

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Direct Telephone Calls to:
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(Fournir les mêmes renseignements et la signature de tout co-inventeur supplémentaire.)

(Supply similar information and signature for third and subsequent joint inventors.)

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<110> RAOULT, Didier
LA SCOLA, Bernard
BIRG, Marie-Laure
FENOLLAR, Florence

<120> DIAGNOSIS OF WHIPPLE'S DISEASE

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<141> 2001-09-24

<150> FR 99/03989
<151> 1999-03-26

<150> FR 99/06679
<151> 1999-05-21

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<110> RAOULT, Didier
LA SCOLA, Bernard
BIRG, Marie-Laure
FENOLLAR, Florence

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